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Evidence for the Phagocytotic Removal of Photoreceptive Membrane by Pigment Cells in the Eye of the Planarian, *Dugesia japonica*

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ABSTRACT—Fine structural changes induced by daily cycles and dark adaptation were investigated in the eye of *Dugesia japonica*. During the daily cycle, an irregular arrangement of microvilli and an accumulation of vesicles in the microvillar area were often observed in animals fixed before dawn. The changes before dawn were enhanced as the period of dark adaptation was prolonged. Pigment cells, which surround the microvillar area, are shown to serve much the same function as the vertebrate pigment epithelium. The pigment cells phagocytose the accumulated vesicles and ingested debris is degraded further into granules and membrane whorls. Internalization by the pigment cells is regarded as one of the mechanisms for the photoreceptive membrane removal in the planarian, *Dugesia japonica*.

INTRODUCTION

The eye of the planarian *Dugesia* is composed of a pigment cup formed by pigment granule-containing cells (pigment cells) and photoreceptor cells whose apical microvilli-bearing processes are enclosed in the pigment cup [1–3]. The fine structure of other planarian eyes has also been well described [4–7]. According to the results of the following studies [4, 8–10], the eye structures are thought not to be stationary, but to change constantly during the normal daily cycle and in conditions of abnormal light- or dark-adaptation. The structural changes may be due to the turnover of the photoreceptive membrane, i.e. addition and removal, as reviewed by Schwemer [11].

Concerning the mechanism of the removal of the photoreceptive membrane, it is well known that the rhabdomeric membrane in the compound eye of many arthropods is internalized by photoreceptor cells via the formation of coated vesicles [12–15]. Also in the planarian *Dalyellia*, Bedini *et al.* [10] postulated an internalization of receptive membrane into the photoreceptor cells, in addition to the drastic changes in the eye structure in daily cycles.

On the other hand, Carpenter *et al.* [3] discussed the possibility that cytoplasmic extensions of the pigment cells, which cover the pupilary opening of the pigment cup, may phagocytose photoreceptive membrane in the eye of the planarian *Dugesia dorotocephala*. Glial cells such as pigment epithelium are known to serve as a removal system in vertebrate eyes [16–18]. Removal of the photoreceptive membrane by surrounding glial cells has also been reported in annelid eyes [19] and in arthropod eyes [20, 21], but generally such the removal is unusual in invertebrate eyes. Therefore, it is interesting to investigate how the pigment cells in the *Dugesia* eye may participate in the membrane turnover system.

I report in this paper that the pigment cells phagocytose remnants of shed photoreceptive membrane in the eye of the *Dugesia japonica*.

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MATERIALS AND METHODS

Specimens of *Dugesia japonica* were collected at Takeda river in the vicinity of Fukui in May and June. The animals live on the underside of rocks which are half buried in the sand. Since it was

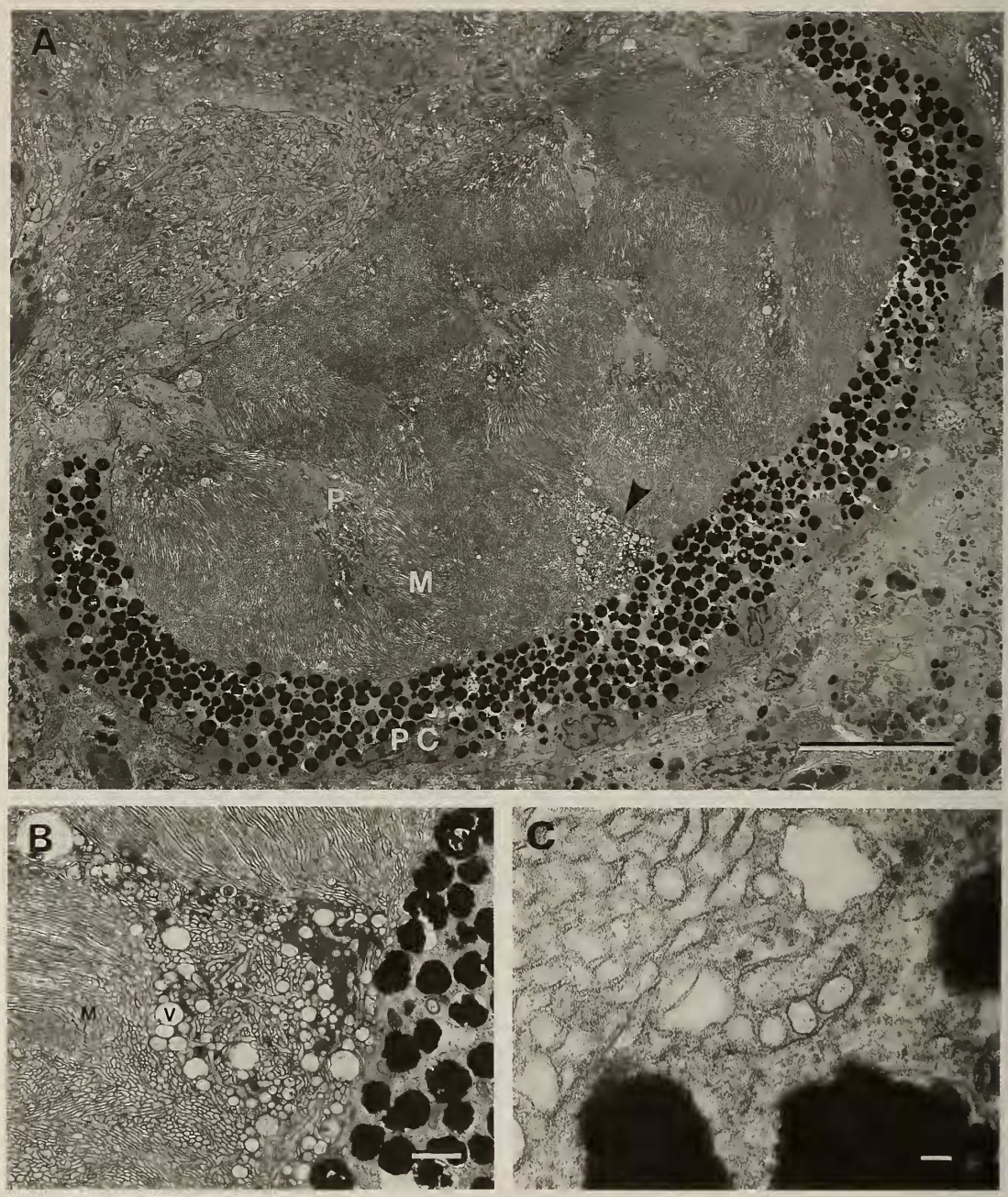

FIG. 1. A: Section through an eye of an animal fixed at 4:00 showing the pigmented eye cup surrounding the microvillar area. (M) microvilli; (PC) pigment cells; (P) microvilli-bearing process of receptor cell. Arrow head indicates the accumulation of vesicles between rhabdomeres and pigment cells. Bar = 10 μm B: Accumulation of vesicles between rhabdomeres fixed at 4:00. The tips of some microvilli were swollen. (M) microvilli; (V) vesicles; Bar = 1 μm. C: Higher magnification electron micrograph of the boundary between the microvillar area and the pigment cells fixed at 4:00. The pigment cells seemed to phagocytose the vesicles. Bar = 0.1 μm.

impossible to perform an accurate measurement of luminous intensity at the places where the animals live, the animals were not maintained in the laboratory but collected at each time they were required for use. Morphological changes depending on daily cycles were investigated in groups of 5 animals, collected under a dim red light and fixed immediately, at 0:00, 4:00, 8:00, 12:00, 16:00 and 20:00 hr. Further groups of five animals collected at 20:00 were dark-adapted for 1 day, 2 days, 4 days, 6 days, 8 days, and 10 days at the same temperature as that of river water, and fixed. All the animals were fixed in 2.5% glutaraldehyde and 0.1 M phosphate buffer (pH 7.4) for 2 hr at 4°C. After trimming the specimens, tissue blocks containing the eyes were postfixed in 1% OsO_4 and 0.1 M phosphate buffer (pH 7.4) for 2 hr at 4°C. The tissue blocks were dehydrated with alcohol series and embedded in Epon 812. Silver thin sections were contrasted with uranyl acetate and lead citrate, and observed with an electron microscope (Hitachi H600).

RESULTS

Morphological changes in the normal daily cycle

The morphology of *Dugesia* eyes has already been described well by MacRae [1], Kishida [2] and Carpenter *et al.* [3]. The eye of *Dugesia japonica* is composed of rhabdomeric photoreceptor cells and pigment cells (Fig. 1A). The pigment cells form a pigmented eye cup surrounding photoreceptive parts of the photoreceptor cells (microvillar area). Microvilli of the photoreceptor cells present an orderly appearance. The microvilli belonging to one cell form a rhabdomere. In the section shown in Figure 1A about 14 rhabdomeres were observed. The microvilli-bearing process contains much smooth endoplasmic reticulum and many mitochondria. Multivesicular bodies smaller than 1 μm in diameter were sometimes observed in the stalk region [3] of the photoreceptor cells. The pigment cup is formed by a single layer of pigment cells. Inner concave surface of the pigment cup possesses few microvilli and is rather flat. Most of spaces in the pigment cell are occupied by membrane bound pigment granules and nucleus.

TABLE 1. Eye volume changes induced by dark adaptation. Area occupied by pigment cells and microvillar area, and their ratios to the total area (pigment cells+microvillar area) were measured on photographs of sections which appropriately contain the optical axis of eyes fixed at 16:00 and eyes dark adapted for 10 days. Volume changes were estimated from these values

	Eyes at 16:00				
	Total (μm^2)	Microvillar area (μm^2)	(/total)	Pigment cells (μm^2)	(/total)
1	3,430	2,270	66.2%	1,160	33.8%
2	3,230	2,180	67.3%	1,060	32.7%
3	2,550	1,590	62.4%	726	37.6%
4	2,740	1,780	64.9%	961	35.1%
Mean	2,990	1,950	65.4%	1,030	34.6%
	Eyes of 10 days dark adaptation				
	Total (μm^2)	Microvillar area (μm^2)	(/total)	Pigment cells (μm^2)	(/total)
1	2,310	989	42.8%	1,320	57.2%
2	1,700	839	49.3%	862	50.7%
3	2,460	1,390	56.4%	1,070	43.6%
4	2,110	1,075	50.9%	1,035	49.1%
Mean	2,150	1,070	50.0%	1,070	50.0%

Investigation of eye structures fixed at 4 hr intervals revealed that some structural changes occur in daily cycles. The rhabdomeric microvilli commonly present an orderly appearance. However, in the eye fixed at 4:00 the microvilli were often irregularly arranged, especially in the area facing the inner concave surface of the pigment cup (Fig. 1A, B). In addition, the tips of some microvilli were swollen (Fig. 1B). Accumulations of vesicles were sometimes observed in extracellular spaces, i.e. between rhabdomeres, in the eyes fixed at 4:00 (arrow head in Fig. 1A, B). The inner concave surface of nearby pigment cells possessed some cytoplasmic extensions extending into the accumulation of vesicles, and the pigment cells seemed to phagocytose the vesicles (Fig. 1C). These structural changes were sometimes observed in the eyes fixed at times other than 4:00, but on a smaller scale.

Morphological changes in dark-adapted condition

After 10 days of dark adaptation, the diameters

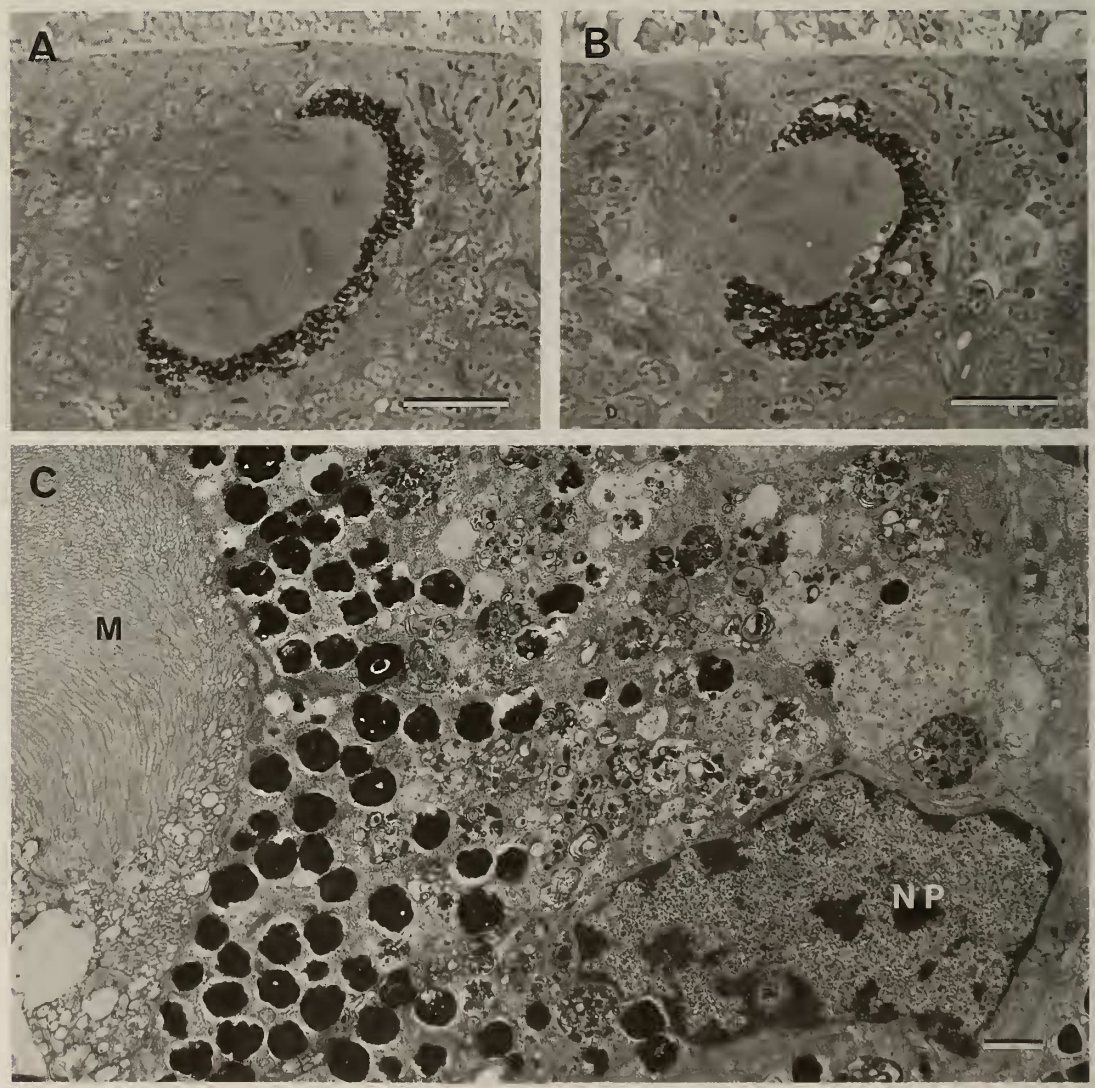

FIG. 2. A: Cross-section showing an eye of an animal fixed at 4:00. Bar=50 μm B: Cross-section showing an eye after 10 days' dark adaptation. Bar=50 μm C: Electron micrograph of pigment cells of an eye fixed after 10 days' dark adaptation. (M) microvilli; (NP) nucleus of pigment cell. Bar=1 μm.

of most eyes became smaller than those of animals maintained in the normal daily cycle (Fig. 2A, B). The dark-adapted eyes had a smaller microvillar area, while the pigment cells were thicker than in normal eyes. In order to make a comparison between the 10 days dark-adapted eyes and the eyes fixed at 16:00, semithin sections containing optical axes of these eyes were made carefully, and the microvillar area and the area occupied by pigment cells were measured (Table 1). During the dark adaptation, microvillar area decreased more than 40%, but the area of pigment cells seemed to increase by a few percent. The pigment cells of dark-adapted eyes contain areas devoid of pigment granules (Fig. 2B), in addition to the perinuclear region. The cellular regions lacking pigment granules contain many vacuoles, which in turn contain membrane debris (Fig. 2C). In normal eyes, pigment granules are lacking only in the perinuclear region (Fig. 2A).

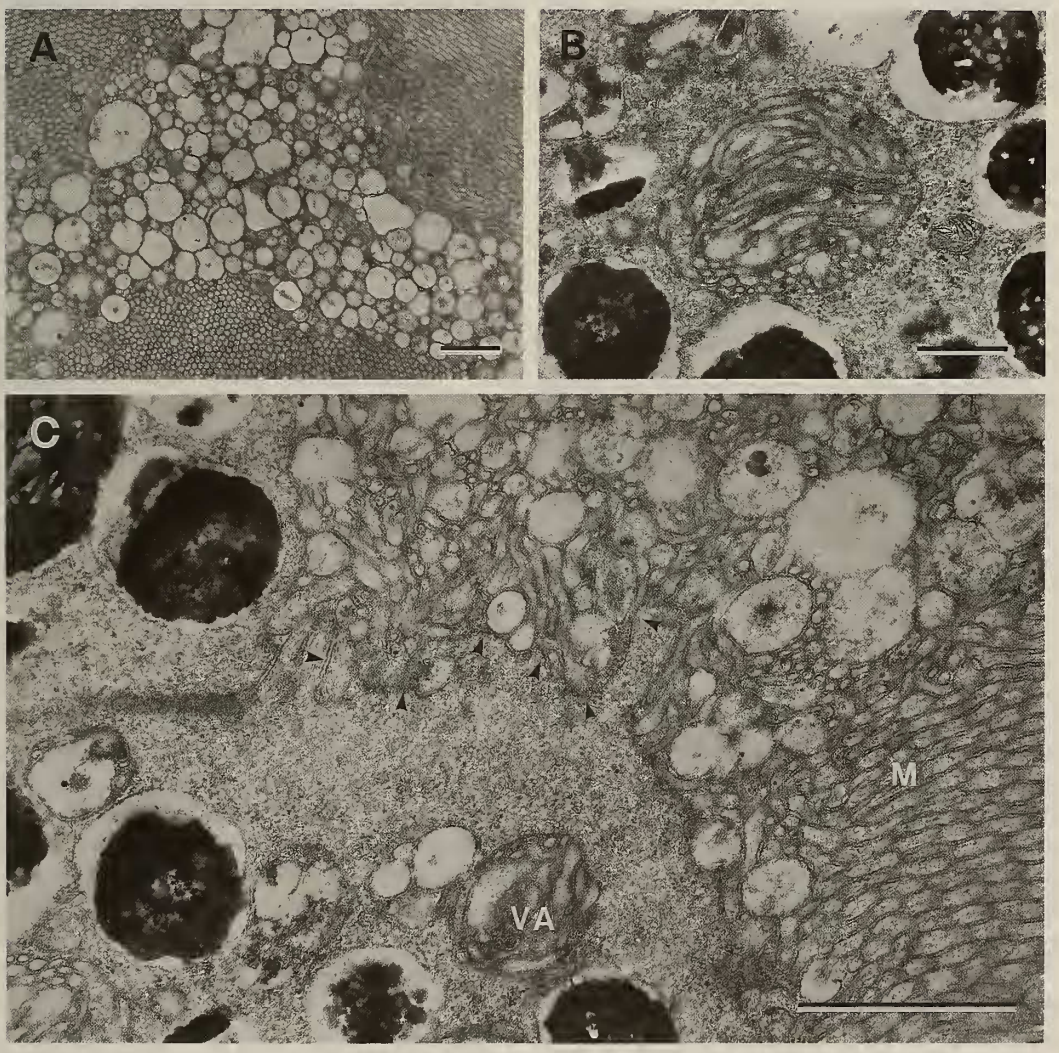

FIG. 3. A: Accumulation of vesicles between rhabdomeres fixed after 8 days' dark adaptation. Bar=1 μm B: Vacuole containing tubular membrane debris observed in the apical portion of a pigment cell fixed after 6 days' dark adaptation. Bar=0.5 μm C: Boundary region between the accumulated vesicles and the pigment cells fixed after 6 days' dark adaptation. Arrow heads indicate the cytoplasmic extensions surrounding the accumulated vesicles. (M) microvilli; (VA) vacuole containing tubular membrane debris. Bar=1 μm.

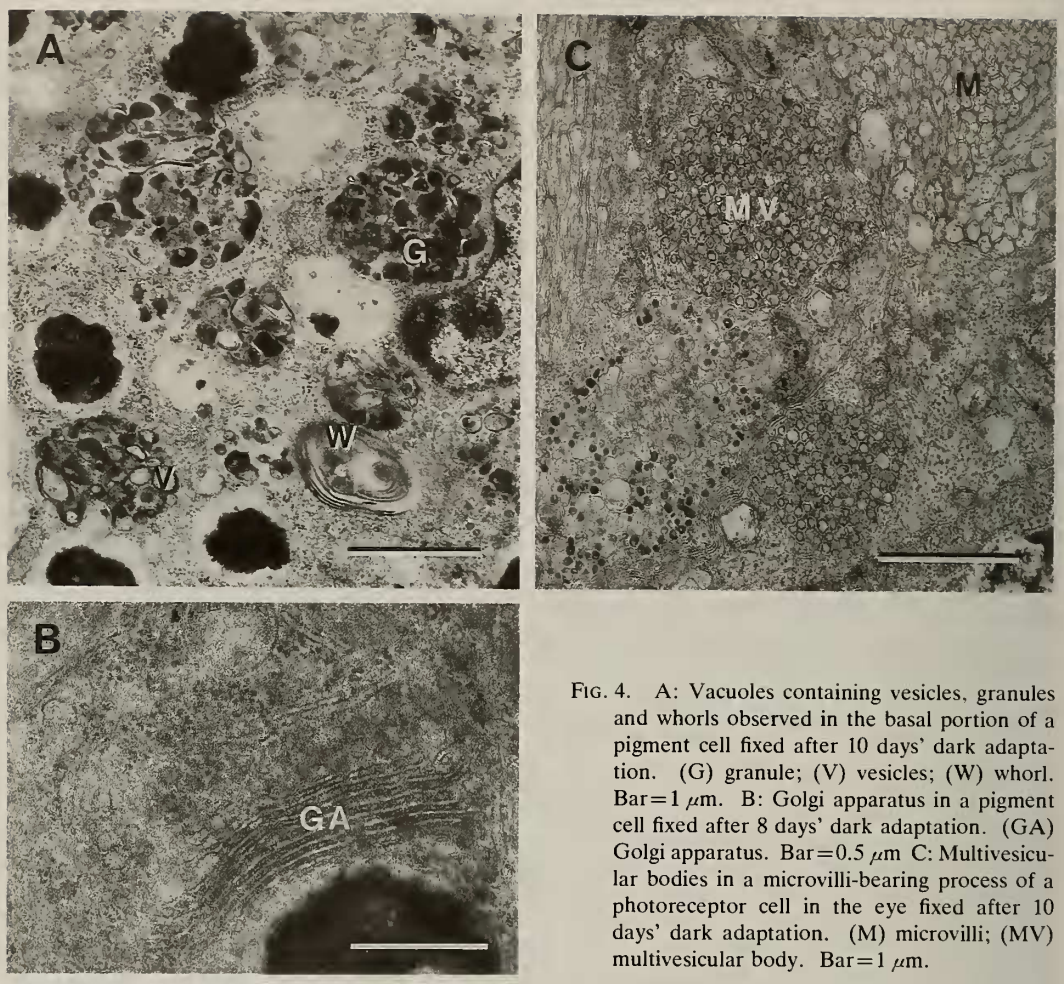

FIG. 4. A: Vacuoles containing vesicles, granules and whorls observed in the basal portion of a pigment cell fixed after 10 days' dark adaptation. (G) granule; (V) vesicles; (W) whorl. Bar=1 μm. B: Golgi apparatus in a pigment cell fixed after 8 days' dark adaptation. (GA) Golgi apparatus. Bar=0.5 μm C: Multivesicular bodies in a microvilli-bearing process of a photoreceptor cell in the eye fixed after 10 days' dark adaptation. (M) microvilli; (MV) multivesicular body. Bar=1 μm.

After longer dark-adaptation, many more microvilli appeared to be irregularly arranged and a considerable number of vesicles were accumulated between the rhabdomeres and pigment cells (Fig. 2C) or between adjacent rhabdomeres (Fig. 3A). The inner surface of the pigment cell, which is ordinarily concave, became convex in the region facing the accumulation of vesicles and possessed cytoplasmic extensions extending into the accumulation of vesicles (arrow heads in Fig. 3C). Vacuoles containing vesicles were often observed in a part of the pigment cells closest to the accumulation of vesicles. Tubular membrane debris, which seem similar to the microvilli of the photoreceptor cells, were also observed in these vacuoles (Fig. 3B and VA in Fig. 3C). The vacuoles observed in that region of the pigment cells closest to the outer surface of the pigment cup contained mainly vesicles, granules and membrane whorls (Fig. 4A). The pigment cells of dark-adapted eyes had Golgi apparatus as shown in figure 4B (Fig. 4B). As one more additional feature of dark-adapted eyes, photoreceptor cells often contained multivesicular bodies larger than 1 μm in diameter, in their microvilli-bearing processes as well as in their stalk region (Fig. 4C).

DISCUSSION

In the eyes of the planarian *Dugesia japonica*, morphological changes in daily cycles and in dark adaptation were investigated with special attention to the pigment cup and microvillar area. An irregular arrangement of photoreceptive microvilli

and an accumulation of vesicles in the perimicrovillar extracellular space were often found in animals fixed before dawn. The eye morphology of the animals used in this study did not show rhythmic circadian changes in total darkness. The morphological changes observed before dawn were enhanced as the period of dark adaptation was prolonged. Therefore, as reported by others [4, 8, 10], light conditions actually have significant effects on photoreceptive membrane structures in the planarian eyes.

Formation of vesicles in the perimicrovillar extracellular space was sometimes regarded as an artifact caused by an inadequate fixation. However, the formation of vesicles is now known to be prevalent in some animals [19, 21, 22], and is regarded as one mechanism of the photoreceptive membrane turnover [11]. In the planarian eye, it is believed that dark adaptation caused a swelling [4] and an irregular wavy appearance in the microvilli [9] as the result of decreased membrane stability and eventual photoreceptor atrophy [8]. Therefore, in the eyes of the planarian *Dugesia japonica,* the accumulation of vesicles which follows the decrease in microvilli is unlikely to be an artifact rather than the result of a biological mechanism. Shedding of microvilli may be initiated by a swelling of the apical edge, leading to an accumulation of vesicles in the perimicrovillar extracellular space.

When the animals are dark-adapted, the decrease in the size of the microvillar area was accompanied by a certain increase in the volume of the pigment cells (Table 1). Such a correlation between the decrease in the microvillar area and the increase in the volume of pigment cells may imply that some amounts of substances are transferred from the microvillar area to the pigment cells. Up until now, the possible phagocytotic activity of pigment cells has been discussed once with respect to the *Dugesia eyes* [3]. The accumulated vesicles in the microvillar area of the dark-adapted eyes seem to be taken up into the pigment cells by phagocytosis (Fig. 3C). Sometimes microvilli may also be taken up into the pigment cells by direct phagocytosis (Fig. 3B). The cellular regions lacking pigment granules contain many vacuoles containing membrane debris (Fig. 2C). These ultrastructural observation undoubtedly support the notion that the substances transferred from the microvillar area to the pigment cells are the vesicles produced by the shedding of microvilli and the microvilli themselves.

Granules and membrane whorls are observed in the vacuoles of the basal half of the pigment cells. Similar prticles are observed in the accessory eye of a giant snail *Achatina fulica* an are thought to be made from shed microvilli membrane [23]. The work of Chamberlain and Barlow [24] supports the idea that membrane whorls are normal breakdown products within the *Limulus* retina. The granules and membrane whorls in the *Dugesia* eye may also be changed from the phagocytosed vesicles.

The process of degradation of the vesicles taken into the pigment cells may be carried out by the action of lysosomal enzymes, such as acid phosphatase (AcPh) [25, 26]. Up to now, I have not seen precisely localized AcPh-deposits within the phagocytic vacuoles and the Golgi apparatus. However, Golgi apparatus may produce this kind of enzyme to degrade the debris of photoreceptive membrane [20]. The reuse of photoreceptive membrane taken into pigment cells has also been discussed by Brandenburger and Eakin [25–27].

After the dark adaptation, multivesicular bodies in the photoreceptor cells increase in number and size. Multivesicular bodies larger than 1 μm were often observed not only in the stalk region but also in the microvilli-bearing processes of the dark adapted photoreceptor cells. The increase in number and size of the multivesicular bodies is also correlated with the decrease in microvillar area. Multivesicular bodies can contribute to that decrease by a resorbence of microvilli membrane, and the multivesicular bodies will be transported from the microvillar area to the perinuclear region of sensory cell. This correlation may imply that the formation of multivesicular bodies is one of the mechanisms of the photoreceptive membrane removal in the eyes of *Dugesia japonica.*

Bedini *et al.* [10] attributed the drastic decrease in microvilli induced by darkness in the *Dalyellia* eye to the resorbence of microvilli into the sensory cells. The resorbence of microvilli into the sensory cells in the planarian *Dalyellia* reminded us of the resorbance of microvilli by the formation of mul-

tivesicular bodies in arthropoda eyes [12–15]. On the other hand, the phagocytosis of pigment cells to remove shed photoreceptive membrane in *Dugesia japonica* reminded us of the phagocytosis of pigment epithelium to remove tipes of rod and cone outer segments in vertebrate eyes [16–18]. There may be two mechanisms by which the photoreceptive membrane is removed from the eye of *Dugesia japonica*. One is the phagocytosis of the pigment cell, i.e. removal by phagocytosis of surrounding glial cells, and the other is the formation of multivesicular bodies in the sensory cells, i.e. removal by resorbence of sensory cells. Swelling of the pigment cells after dark adaptation is induced by the phagocytosis of shed photoreceptive membrane in the microvillar area. Although the increase in the volume of pigment cells was small and did not completely compensate the decrease in the microvillar area, the greater volume of membrane debris would be phagocytosed and digested by the pigment cells. Therefore, even if the formation of multivesicular bodies may be a potential explanation for the decrease of the microvillar area, the phagocytosis by pigment cells must be also regarded as one of the mechanisms for the photoreceptive membrane removal in the planarian *Dugesia japonica*. It is very interesting to know that the two mechanisms for photoreceptive membrane removal prevailing in vertebrate and invertebrate coexist in planarian eyes.

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